Diminished ovarian reserve and adverse reproductive outcomes

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Chapter 3

Low oocyte yield during IVF treatment and the risk of a trisomic pregnancy

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Abstract

A low number of antral follicles may result in the selection of suboptimal oocytes that are prone to meiotic errors. The aim of this case-control study was to evaluate whether IVF-treated women with low oocyte yield (defined as ≤ 3 oocytes retrieved after controlled ovarian stimulation) are at an increased risk of a trisomic pregnancy. Data were obtained from Danish and Dutch medical registries between 1983 and 2011. Analyses were performed in 105 cases and 442 controls matched by age and year of IVF treatment. Cases were women with a trisomic pregnancy (trisomies 13, 18 or 21) resulting from fresh IVF treatment and confirmed by karyotyping. Cases were included regardless of pregnancy outcome. Controls were women with a live born child without a trisomy, resulting from fresh IVF treatment. Low oocyte yield was observed in 6.6% (29/440) of the women, of which 8.4% (7/83) were cases and 6.2% (22/357) controls. Low oocyte yield in IVF treatment was not associated with a higher risk of trisomic pregnancy (OR 1.43, 95% CI 0.64-3.19). Stratification for female age, adjustment for history of ovarian surgery, and GnRH protocol used did not change the results.

Key words: oocyte; ovarian reserve; trisomy; IVF.

Key message: Our results indicate that a low oocyte yield in response to ovarian hyperstimulation during IVF treatment is not associated with a higher risk of having a trisomic pregnancy.

Introduction

Female age and the number of retrieved oocytes are important determinants of success in *in vitro* fertilisation (IVF) treatment with controlled ovarian stimulation (Sunkara *et al.*, 2011; Oudendijk *et al.*, 2012; Polyzos *et al.*, 2014). In women of advanced age, oocytes are more susceptible to meiotic errors leading to aneuploid embryos, which increase the risk of trisomic pregnancies (Fragouli *et al.*, 2011; Eichenlaub-Ritter, 2012; Harton *et al.*, 2013). Likewise, low oocyte yield, i.e., ≤ 3 oocytes retrieved after controlled ovarian stimulation in IVF treatment (Ferraretti *et al.*, 2011), has been hypothesized to be associated with higher aneuploidy rates. Low oocyte yield is often the result of having a low number of antral follicles available (Younis *et al.*, 2005). The limited pool hypothesis states that a low number of antral follicles results in the selection of suboptimal oocytes that are prone to non-disjunction during meiosis (Warburton, 1989). Thus, women with a low oocyte yield are hypothesized to have an increased risk of aneuploidy, irrespective of their age.

In a previous study in a Dutch IVF cohort, we could not confirm or reject this hypothesis. There was a non-significant increased risk of a trisomic pregnancy for women who had ≤ 3 oocytes were retrieved (OR 2.72 96%CI 0.69-10.69) and a significant increased risk when ≤ 4 oocytes were retrieved (OR 3.97 95%CI 1.37-11.51). Although an increased risk was observed for both cut-offs, for the most common definition of poor response (≤ 3 oocytes), it was not significant (Haadsma *et al.*, 2010b). Additionally, the confidence intervals were quite large, preventing a definite conclusion. Therefore, we replicated our study in a different and larger cohort of Danish women. In order to increase the generalizability of our results and provide an estimation of the association between oocyte yield and trisomy risk, we combined the data from the Danish cohort and the Dutch cohort previously published. This study aimed to determine whether IVF-treated women with a low oocyte yield are indeed at an increased risk of a trisomic pregnancy.

Material and Methods:

Study design

We performed a matched case-control study within a nationwide Danish IVF cohort (n=379). Additionally, the Danish cohort was combined with a previously published Dutch case-control study within a nationwide cohort (n=168) of IVF-treated women (conventional IVF or IVF with intracytoplasmic sperm injection (ICSI)). Therefore this study contains analyses of three datasets: the Danish cohort, the Dutch cohort (re-

analysed with current statistical methods to standardize results) and the Danish and Dutch combined cohort.

The Danish cohort

Data for the Danish cohort were selected from three Danish nationwide registers: the Hospital Discharge Register, the IVF Register (paper-based reporting for the period of 1999-2005 and electronic reporting for the period of 2006-2010) and from the Cytogenetic Central Register. These registries cover all Danish IVF cycles since records are compulsory and required by law in Denmark. Women who had controlled ovarian hyperstimulation with either human menopausal gonadotropin (hMG) or recombinant follicle stimulating hormone (rFSH) were included. Women who took clomiphene citrate during their IVF treatment were excluded as were women without information on their type of medication. Each woman could contribute to the data with one treatment cycle only, independent of the cycle number. Pregnancies resulting from frozen embryo transfer or oocyte donation were not included. Cases were women with a trisomic pregnancy (trisomies 13, 18 or 21) resulting from fresh IVF treatment. Trisomic pregnancies were either identified after elective prenatal diagnosis or at the identification of ultrasound abnormalities, intra-uterine death, still birth or after birth, and confirmed by karyotyping. Trisomies caused by a chromosomal translocation were not included, since these may result from parental balanced chromosomal translocations rather than being associated with maternal age. Cases were included regardless of the pregnancy outcome (termination, intra-uterine death, stillbirth or live born child). Controls were selected independent of oocyte yield and included women with a live born child without a trisomy, resulting from fresh IVF treatment. Matching criteria were female age at start of IVF treatment and year of IVF treatment. A maximum of four controls were selected for each case, if available after applying matching criteria. Data were structured and analysed in clusters, i.e., grouping cases and their matched controls, since observations within clusters are correlated and cannot be regarded as independent, while observations between clusters are independent (Halekoh *et al.*, 2006) . This study was performed with permission from the Danish National Board of Health (file number 6-8011-950/1) for the Danish cohort.

The Dutch cohort

The Dutch cohort has been described previously (Haadsma *et al.*, 2010b). Briefly, it is a retrospective nationwide cohort study (OMEGA project) of infertile women visiting one of the twelve Dutch IVF centres between 1983-1995. Each woman could contribute to the data with one treatment cycle only, independent of the cycle

number. Cases were women with a trisomic IVF-pregnancy (trisomies 13, 18 and 21, confirmed by karyotype, excluding translocation-based trisomies) regardless of pregnancy outcome (termination, intra-uterine death, stillbirth or live born child). Controls were selected independent of oocyte yield and included women with a live born child after IVF, without a trisomy. Matching criteria were female age at start of the IVF treatment, year of IVF treatment and fertility centre. Each case was matched to five controls and data were structured and analysed in clusters. For this cohort, two cases resulting from spontaneous pregnancies which occurred within one year after the IVF treatment were included along with their matched controls to maximize sample size. The number of oocytes from the treatment was taken as a proxy of their ovarian reserve status.

The institutional review boards of the participating IVF centres approved the study protocol for the Dutch cohort, and all patients gave written informed consent and permission to search their medical files.

The Danish and Dutch combined cohort

The Danish and Dutch cohorts were combined and analysed as one cohort. The structure of clusters of cases and controls was maintained. The analyses were performed stratifying the data by site (Dutch or Danish).

Statistical methods

For the current study, the relationship between oocyte yield and risk of trisomy was analysed in three different ways, i.e., with oocyte yield as a continuous outcome, as a dichotomous outcome (≤3 as low oocyte yield or ≥ 4 oocytes as normal oocyte yield) and with spline regressions. The cut-off of ≤ 3 oocytes was defined as such according to current clinical recommendations (Ferraretti *et al.*, 2011). Since the defined cut-off value for low oocyte yield may not correlate with the biological association between oocyte number and trisomy risk, we also performed restricted cubic spline regressions to explore the possibility of a non-linear relation between the number of oocytes and trisomy risk. To evaluate whether a low oocyte yield increases the risk of having a trisomic pregnancy, we used generalized estimating equations (GEE). This statistical method accommodates the nested structure of the data, i.e. the clusters of cases and their matched controls, even if cluster size varies, and it provides unbiased estimations when missing values are missing at random (Little and Rubin, 2002). We assumed a fixed non-zero correlation within clusters (exchangeable working correlation matrix), i.e., cases and controls within the clusters are not independent due to the grouping by similar characteristics.

Subanalyses were performed with factors that could affect the number of oocytes including stratification by female age (younger or older than 40 years), adjustment for use of a gonadotropin-releasing hormone (GnRH) agonist or antagonist protocol and history of ovarian surgery prior to IVF treatment.

Splines were calculated using Stata LC version 11 (Statacorp LP, College Station, Texas, USA). SPSS Statistics, version 20, was used for all the other analyses (IBM Corporation, Armonk, NY, USA). A p-value of less than 0.05 was considered statistically significant.

Results

The results of the Danish cohort, Dutch cohort and Danish and Dutch combined cohort are described below. Table I shows the characteristics of the respective cohorts and table II shows the odds ratios (ORs) for a trisomic pregnancy associated with oocyte yield.

Danish cohort

The Danish cohort included a total of 408 women, of which 85 were cases and 323 matched controls. There was no statistical age difference between cases and controls (p-value=0.52), asserting that the matching was successful. Eight cases and 21 controls with clomiphene citrate use or missing information on medication were excluded, leaving 379 women (77 cases and 302 controls) for analysis. In total, 53 cases had four controls matched; 17 cases had three controls, five cases had two controls and one case had one control. One case and 28 controls were not matched after exclusions and these clusters were maintained in our analysis since the statistical method accounted for missing values and clusters of different sizes.

The main causes of infertility were male factor (30.9%) and unexplained infertility (22.2%). Of all women, 28% (107/379) had an unknown number of oocytes retrieved, of which 28.6% (22/77) were cases and 28.1% (85/302) controls. We performed a missing values analysis and which showed no specific pattern in the missing values. There was no statistical difference between missing data and available data for the number of oocytes for age (37.1 versus 36.4 respectively, p=0.15 Mann-Whitney U test), ovarian surgery (8.4% versus 10.3% p=0.58, Pearson's Chi Square test) and causes of infertility (male factor p=0.14, unexplained p=0.37, Pearson's Chi Square test) validating the assumption of missing at random.

In the Danish cohort, a low oocyte yield was not associated with a higher risk of having a trisomic pregnancy after IVF (see table II). Stratification for female age younger or older than 40 years did not change the results. Women with a history of ovarian surgery before IVF treatment, despite having decreased ovarian volumes, did not have a higher risk of a trisomic pregnancy compared to women without previous surgery.

In the Danish dataset, 268 women (55 cases and 213 controls) used a GnRH agonist and 66 women (15 cases and 51 controls) a GnRH antagonist. The mean number of oocytes retrieved in women who used agonists was significantly higher than in the women who used antagonists (9.9 ± 4.6 and 7.4 ± 3.8 respectively, p-value < 0.01), but adjusting for the use of either a GnRH agonist or antagonist did not change our results (OR 0.99, 95% CI 0.79-1.24).

The Dutch cohort

The results for the Dutch cohort have been reported previously (Haadsma *et al.*, 2010b).. Table II shows the main results of the Dutch data re-analysed with current statistical methods. There was no statistical association between low oocyte yield and trisomic pregnancy. Women with a history of ovarian surgery before IVF treatment did not have a higher risk of a trisomic pregnancy.

Since the Danish cohort included only pregnancies resulting from IVF, sensitivity analysis to maximize comparability with the Danish cohort was performed excluding the Dutch spontaneous pregnancies (2 cases and their 10 matched controls). Results for the risk of miscarriage for low oocyte yield did not change materially (OR 1.02, 95% CI 0.99-1.05).

The Danish and Dutch combined cohort

In total, 547 women were included in the combined analyses (105 cases and 442 controls). The number of retrieved oocytes was unknown in 19.6% (107/547) of the women. Low oocyte yield in IVF treatment was not associated with the risk of a trisomic pregnancy. The results were similar when analyses were stratified for female age (younger or older than 40 years). A history of ovarian surgery before IVF treatment was not associated with a higher risk of having a trisomic pregnancy. Sensitivity analysis excluding the Dutch spontaneous pregnancies (2 cases and their 10 controls) did not change the results for the risk of miscarriage for low oocyte yield (OR 1.26, 95% CI 0.81-1.97).

Table I. Characteristics of the Danish cohort, Dutch cohort, and Danish and Dutch combined cohort.
Footnote: Previously reported data (Haadsma et al., 2010)

	Danish Cohort n=379		**Dutch Cohort[a] n=168**		**Danish and Dutch combined cohort n=547**	
	Cases n= 77	**Controls n=302**	**Cases n=28**	**Controls n=140**	**Cases n=105**	**Controls n=442**
Age (yrs)	36.9 ± 3.8	36.5 ± 3.7	35.9±3.3	36.2±3.2	36.7 ± 3.7	36.4 ± 3.6
Trisomy total						
13	4 (5.2%)	-	1 (3.6%)	-	5 (4.5%)	-
18	17 (22.1%)	-	3 (10.7%)	-	20 (18.2%)	-
21	56 (72.7%)	-	24 (85.7%)	-	80 (77.3%)	-
No. of oocytes retrieved						
Mean; SD	9.7 ± 4.8	9.4 ± 4.6	8.5±6.0	9.7±5.5	9.3 ± 5.2	9.5 ± 5.0
Low oocyte yield						
Yes (≤ 3)	3(5.5%)	13(8.8%)	4 (14.3%)	9 (6.4%)	7 (8.4%)	22 (6.2%)
No (≥ 4)	52 (94.5%)	204 (94.0%)	24 (85.7%)	131 (93.6%)	76 (91.6%)	335 (93.8%)
Ovarian surgery before IVF						
yes	10 (13.0%)	27 (8.9%)	5 (17.9%)	8 (5.7%)	15 (14.3%)	35 (7.9%)
no	67 (87.0%)	275 (91.1%)	23 (82.1%)	132 94.3%)	90 (85.7%)	407 (92.1%)

Table II. Odds Ratio for trisomic pregnancy associated with oocyte yield: Danish cohort, Dutch cohort and Danish and Dutch combined cohort.
*Footnote: abbreviations: OR odds ratio, CI confidence interval. *Only 15 women with more than 40 years of age.*

	Danish cohort	**Dutch cohort**	**Danish and Dutch combined cohort**
	OR, 95% CI	OR, 95% CI	OR,95% CI
Oocyte yield	1.00, 0.99-1.01	1.00, 0.99-1.00	1.00, 0.97-1.03
Low oocyte yield			
≤ 3 oocytes	1.00, 0.97-1.03	1.02, 0.99-1.05	1.43, 0.64-3.19
≥ 4 oocytes	reference	reference	Reference
Subanalysis- stratified data			
Women < 40 years	0.99, 0.93-1.06	1.11, 0.81-1.54	1.13, 0.65-1.97
Women ≥ 40 years	1.28, 0.58-2.83	*	2.16, 0.43-10.8
Ovarian surgery before IVF			
Yes	1.02, 0.88-1.20	1.05, 0.98-1.13	1.47, 0.89-2.44
No	reference	reference	Reference

Figures I, II and III show spline regressions for the Danish cohort, the Dutch cohort and the Danish and Dutch combined cohort. In the Danish cohort a low number of oocytes retrieved was not associated with a higher probability of a trisomic pregnancy (p = 0.99). Although visual inspection of the spline curves for the Dutch and the Danish and Dutch combined cohorts suggested an increased probability of a trisomic pregnancy at lower numbers of oocytes retrieved, the non-linear association was not statistically significant (p = 0.19 and p = 0.40, respectively).

Figure I: Spline regression for the Danish cohort

Footnote: Spline regression is a technique to investigate non-linear relationships between a determinant (number of oocytes) and an outcome (risk of trisomy). The figure shows restricted cubic spline regression with the probability of having a trisomic pregnancy calculated for each of the values of the number of oocytes retrieved (see dots,•) . A dot may represent one or more cases or controls. In case of more than one case with the same number of oocytes, the average outcome is presented (e.g., 0.25 for cases with one oocyte retrieved). The regression line for each spline segment for number of oocytes is connected with smoothed transitions (red line). The grey area represents the 95% CI.

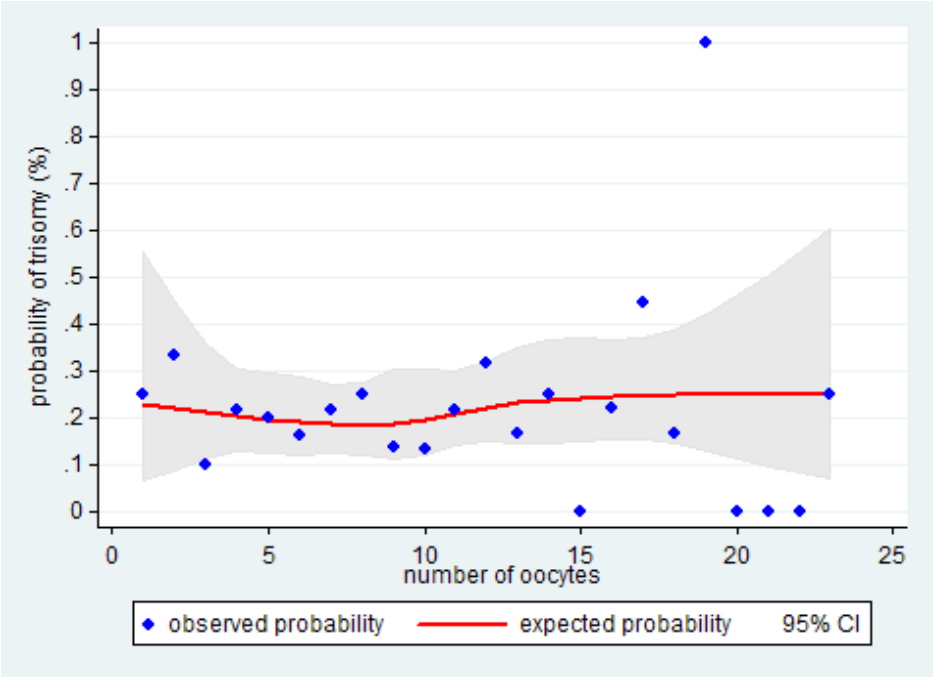


Figure II: Spline Regression for the Dutch cohort. See footnote of figure I

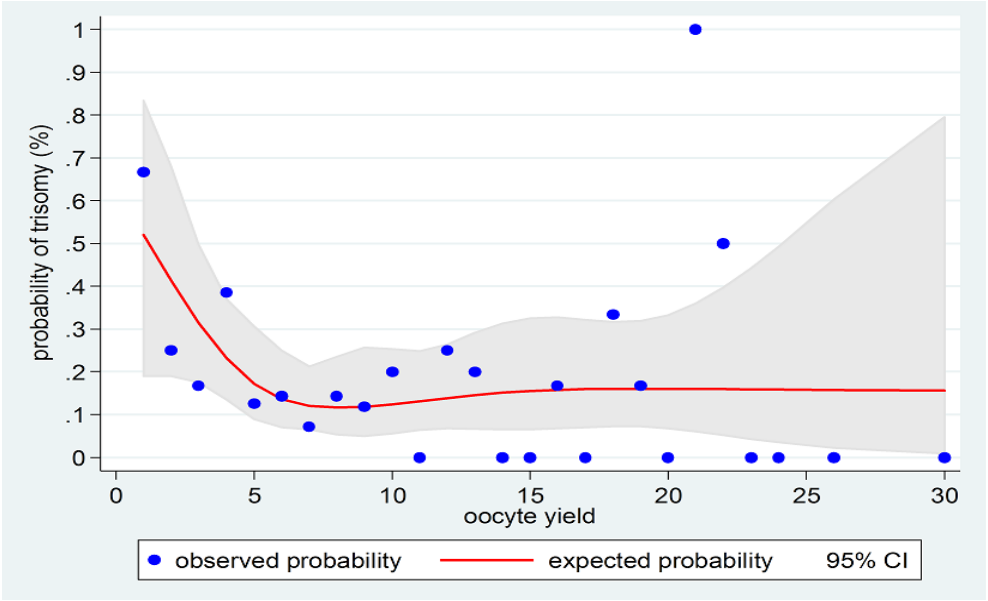
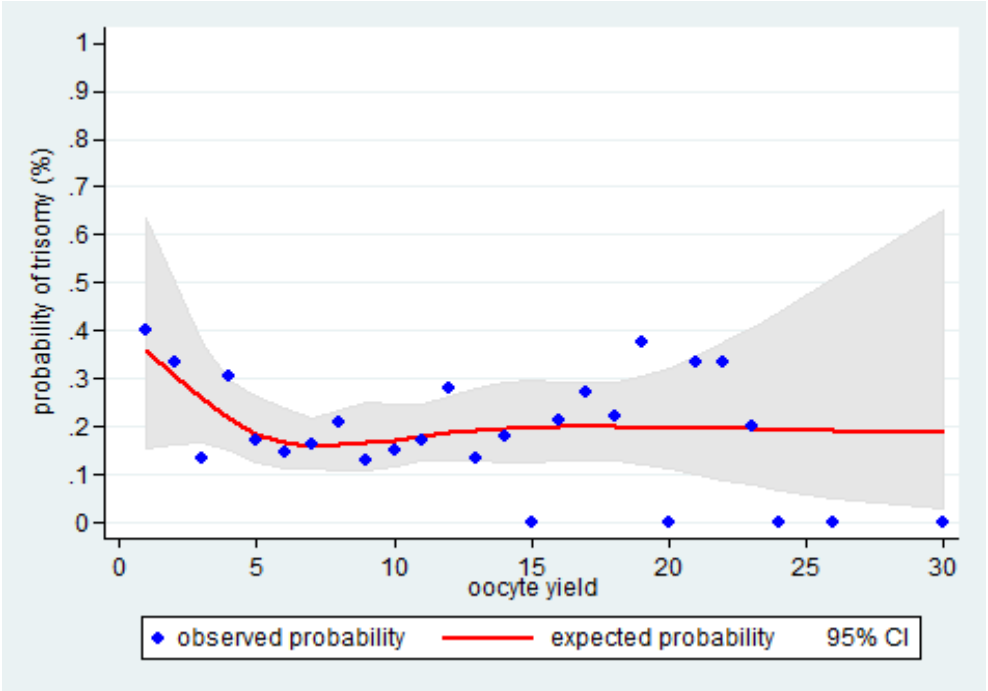


Figure III: Spline Regression for the Danish and Dutch combined cohort. See footnote of figureI



Discussion

Our results indicate that a low oocyte yield in IVF treatment is not associated with an increased risk of having a trisomic pregnancy, irrespective of female age. Our results do not support the hypothesis that a lower number of follicles available after IVF with controlled ovarian stimulation is associated with higher aneuploidy rates.
The strengths of our study include its age-matched case-control design. Trisomy risk is strongly associated with maternal age. Our matched case-control design minimises residual age-related confounding. The statistical method we used is appropriate for clustered data and provided precise results, as shown by the narrow confidence intervals.

The limitations of our current study are the lack of information on cycle number and on ovarian reserve test results. Moreover, we lacked information on the dose of gonadotropins used for controlled ovarian stimulation and on cycle cancellation policies. We have, however, no reason to assume that dosages differed between cases and controls. The data were matched by age, which is often used in clinical practice to determine a starting stimulation dose. Therefore it can be expected that within clusters the dosages of stimulation for cases and controls are correlated. For most women, smoking status and BMI were not available, so we could not adjust our analyses for these factors. Finally, this study did not include early pregnancy losses, in which non-viable trisomies occur more frequently. Therefore results of our study may be applicable to clinically confirmed pregnancies only.

This study analysed the limited pool hypothesis in a population of women who received controlled ovarian stimulation. Women with a low number of available antral follicles who achieve a spontaneous pregnancy may differ from women who conceived after controlled ovarian stimulation and IVF (Kline *et al.*, 2011). In controlled ovarian stimulation treatment, less viable follicles and oocytes may be rescued preceding ovum pick up and selection of oocytes in the laboratory may further bypass natural selection that would have occurred in spontaneous ovulation followed by a conception. Thus, generalizability of our results to women who achieved spontaneous pregnancy may not be straightforward.

The Danish and Dutch combined cohort included patients who had IVF treatment between 1983 and 2011. Over these decades, techniques in oocyte collection did not change substantially (Kovacs, 1999; Wang and Sauer, 2006) and patients treated by newer techniques, such as cryopreservation or gamete donation, were not included in this study. Ovarian stimulation protocols, on the contrary, have changed over time, with a tendency to the use of lower dosages of stimulation (Macklon *et*

al., 2006). Therefore cases and controls were matched by year of treatment as well. Nevertheless, randomized control trials indicate little or no clinical benefits when higher dosage regimes are given to poor responders (Siristatidis and Hamilton, 2007) and differences in protocols have been shown to have no effect on aneuploidy rates (Gianaroli *et al.*, 2010). In our analysis, we adjusted for the use of GnRH antagonists, which are known to decrease the average number of oocytes in IVF (Al-Inany *et al.*, 2011), but this did not change the risk of a trisomic pregnancy.

Our overall current findings differ from our previously published results (Haadsma *et al.*, 2010b). This may be due to differences in the methods and the characteristics of the two cohorts. The Dutch cohort previously published was analysed with multivariate conditional logistic regression and a higher risk of a trisomic pregnancy was observed in women who had less than 3 or 4 oocytes retrieved (OR 2.72, 95% CI 0.69-10.69 and OR 3.97, 95% CI 1.37-11.51 respectively). This was no longer observed in the current study when the Dutch data were analysed with GEE. This could indicate that our previous results had some sparse-data bias, i.e. an overestimation of results when small samples are analysed with conditional logistic regression (Greenland *et al.*, 2000). The GEE analysis includes maximum likelihood to correct for bias (Greenland *et al.*, 2000).

The Dutch and Danish cohorts were comparable in the distribution of variables relevant for this analysis, including female age and the proportion of low oocyte yield. Overall, the characteristics of child-bearing women in the Netherlands and Denmark, such as fertility rates and age at first pregnancy, are comparable (Euro-Peristat, 2013), and the overall prevalence of trisomies in both countries has remained stable over the study period (Loane *et al.*, 2011). However, there are also differences between the two cohorts. Denmark has one of the highest rates for IVF treatment utility (Kupka *et al.*, 2014; Ishihara *et al.*, 2015), and therefore Danish women might have had a shorter duration of infertility at the start of IVF treatment (Pinborg *et al.*, 2011). A longer duration of infertility might play a negative role in pregnancy rates after IVF treatment (Axmon and Hagmar, 2005).

Low oocyte yield after controlled ovarian stimulation in IVF is considered to reflect diminished ovarian reserve. However, there is no agreement in the literature on the definition of diminished ovarian reserve nor on which of its features are associated with poor outcomes (Cohen *et al.*, 2015). Previous studies showed inconsistent results regarding the relation between various parameters reflecting diminished ovarian reserve (e.g. abnormal ovarian test results and previous ovarian surgery) and clinical outcomes (e.g. miscarriages and trisomic pregnancies) (van Montfrans *et al.*, 1999, 2002; Freeman *et al.*, 2000; Kline *et al.*, 2004, 2011; Grande *et al.*, 2014). These

differences could be due to variation in definitions of low oocyte yield or diminished ovarian reserve used. Nevertheless, there is consensus on poor responders in IVF, as defined by the Bologna criteria (Ferraretti *et al.*, 2011). Poor responders fulfilling these criteria are considered to have diminished ovarian reserve. The Bologna criteria define poor responders as women with two previous low oocyte yields (cycles cancelled or ≤ 3 oocytes) under optimum ovarian stimulation or women with at least 2 out of the 3 features: (i) maternal age ≥ 40 years, (ii) a previous low oocyte yield (cycles cancelled or ≤ 3 oocytes) and (iii) abnormal ovarian reserve test. These criteria better identify women with diminished ovarian reserve (Ferraretti and Gianaroli, 2014; Busnelli *et al.*, 2015) compared to women with low oocyte yield in one IVF cycle only.

Despite the large sample size of the Danish cohort and the Danish and Dutch combined cohort, the number of cases with low oocyte yield is still too small for final conclusions. Ideally, a prospective larger case-control study, focussing on poor responders as defined by the Bologna criteria, would determine whether this specific group is at an increased risk for trisomic pregnancies. However, collecting sufficient data for such a cohort of IVF-treated women will be a great challenge for an event as rare as trisomic pregnancy, especially if information on ovarian reserve tests for all patients with a low oocyte yield has to be included.

To conclude, our results are reassuring and indicate that low oocyte yield is not associated with an increased risk of a trisomic pregnancy. A small increase of risk may exist, but it would require a larger study to prove this.

Authors' roles

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Hoek A: study design, execution, analyses, manuscript drafting and critical discussion

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